d) assaying the ability of the bacteriophage to bind the target nucleic acid sequence and selecting the bacteriophage demonstrating superior binding characteristics.

Please add the following new claim:

32. (New) The method of claim 3, wherein a plurality of overlapping quadruplets are selected within the target sequence.

REMARKS

Claims 1-30 are presently pending. Claims 25-30 are withdrawn from consideration. Claim 31 has been cancelled. Claims 1-24 have been examined on the merits and stand variously rejected. Support for selecting a quadruplet within a target sequence as recited in amended claims 1 and 3 is provided by *e.g.*, p. 35, lines 10-14. Support for new claim 32 (overlapping quadruplets) is provided at *e.g.*, p.33, line 10 and Fig. 1.

The paragraph numbering of the office action is used in responding to the Examiner's comments.

2. The Examiner has clarified and modified the original restriction requirement. Applicants maintain election of Group I. The modified requirement is traversed insofar as it separates the Group II claims. It is noted that the present case is a §371 application of a PCT. The correct standard for a §371 application is unity of invention, under which a "group of inventions is considered linked to form a single general inventive concept where there is a technical relationship among the inventions that involves at least one common or corresponding special technical feature" (MPEP §1893.03(d)). Here, the Group II claims reference and incorporate all elements from claim 3 of the Group I claims (see step (a) claim 25 "preparing a nucleic acid binding protein by the method of claim 3...". Therefore, the Group II claims include the same

technical features as the Group I claims. Accordingly, it is submitted that there is unity of invention between Group I and Group II claims and these Groups be recombined for prosecution.

Restriction between Group III and Groups I and II is also traversed on the basis that the asserted materially different process in which nucleic acids of Group II can be used, a hybridization assay, is merely a throw-away utility. It has not been explained why one would use a nucleic acid encoding an engineered zinc finger protein in a hybridization assay or what kind of target one could hope to identify using such a nucleic acid.. In the absence of a real utility of the Group III claims that is not shared with Groups I and II, Group III should not be restricted.

4. Claims 4-11 are objected to for alleged failure to recite SEQ ID NOS. It is noted claim 4 does recited a SEQ ID NO. added by an amendment of December 28, 2000. (Applicants note that three preliminary amendments have been filed on December 28, 2000, June 11, 2001 and November 5, 2001, and the Examiner is asked to check that all have been entered. Claim 9 has been amended herein to recite SEQ ID NOS. Further, the remaining claims specify species falling within the genus represented by the SEQ ID NO. of claim 4. SEQ ID NOS are not required for such variants (MPEP 2422.03 "It is generally acceptable to present a single, general sequence in accordance with the sequence rules and to discuss and/or claim variants of that general sequence without presenting each variant as a separate sequence in the 'Sequence Listing.'")

5. As requested, an abstract is provided on a separate sheet attached to this response.

6. Applicants provide copies of the previously submitted information disclosure statements together with proof of receipt of the original filings including references. In view of the attached proof of receipt, it appears that the references were lost within the PTO. Therefore, applicants submit that the presently submitted

information disclosure statements should be considered with effect from the original submission date under *e.g.*, 37 CFR §1.97(f) ("If a bona fide attempt is made to comply with §1.98, but part of the required content is inadvertently omitted, additional time may be given to enable full compliance"). Due to the voluminous nature of the references, and the fact that two complete sets of the references have already been lost in the PTO, and a further set has been provided in copending application 09/424,488, which is being handled by the present Examiner, applicants suggest that the Examiner review the references present in the copending application. However, if the Examiner is unable to access the references in this way, she is asked to call the undersigned for supply of a further set.

8. Claims 1-24 stand rejected under 35 US §101 as allegedly directed to non-statutory subject matter, for allegedly failing to recite positive methods steps. It is respectfully submitted that the claimed methods relate to methods for selecting amino acid residues at particular positions with a zinc finger, based on the nucleotide sequence of the target site to which the zinc finger binds, and that it would be apparent to one of skill in the art that the claims are directed to such methods of selection[1]. Nonetheless, and without conceding the correctness of the Office's position, the claims have been amended to recite a series of method steps. In light of these amendments, applicants believe that the §101 rejection can properly be withdrawn.

9-10. Claims 1-24 stand provisionally rejected on the basis of same invention double patenting over claims 1-23 and 27 of copending application 09/424,488 on the basis that the claims have similar language, scope, wording and subject matter. This rejection is respectfully traversed.

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[1] Although the selection rules themselves are novel, and would not be apparent to one of skill in the art.

"If there be any substantive difference and not merely a difference in language then the inventions are not the same no matter how small or how obvious those difference may be." In *re White*, 60 USP 417, 419 (CCPA 1969). Here, a comparison of the rules recited in claim 3 of the present case and '488 is shown below:

		Present	'488
base 4 (5' most base)	**G**	Arg +6 Lys +6	Arg +6 Ser +6/Asp ++2 Thr +6/Asp ++2
	A	Glu +6 Asn +6 Val +6	Gln +6**
	T	Ser +6 Thr +6 Val +6 Lys +6	Ser +6/Asp ++2 Thr +6/Asp ++2
	C	Ser +6 Thr +6 Val +6 Ala +6 Glu +6 Asn +6	++2 not Asp
base 3	**G**	His +3	His +3
	A	Asn +3	Asn +3
	T	Ala +3* Ser +3 Val +3	Ala +3* Ser +3 Val +3
	C	Ser +3 Asp +3 Glu +3 Leu +3 Thr +3 Val +3	Ser +3 Asp +3 Glu +3 Leu +3 Thr +3 Val +3

base 2	**G**	Arg –1	Arg –1
	A	Gln -1	Gln –1
	T	His –1 Thr -1	Asn –1 Gln –1
	C	Asp –1 His -1	Asp –1
base 1 (3' most base)	**G**	Glu +2	Asp +2
	A	Arg +2 Gln +2	+2 not Asp
	T	Ser +2 Thr +2	Ser +2 Thr +2
	C	Asn +2 Gln +2 Arg +2 His +2 Lys +2	+2 not Asp

* small residue at -1 or +6

** ++2 is not Asp

The Examiner can readily see that although there are some similarities, there are also many differences. The existence of any such difference is sufficient to defeat a same-invention double patenting rejection. Therefore the rejection should be withdrawn.

11. Claims 1-24 stand rejected under 35 USC §112, second paragraph, as allegedly indefinite for several reasons that will be addressed in turn.

First, the office action states that the subject matter of claim 1 is not clearly defined, as it does not recite method steps, but merely recites a rule which defines 1 amino acid in an otherwise undefined protein. In response, the claim has been amended to make clear that the recited rule is part of a larger process for determining the sequence

of the recited nucleic acid-binding zinc finger. This is consistent with the teaching of the specification, whereby disclosed rules of design can be used in combination with other design rules (see paragraph bridging pp. 6-7), with molecular modeling (specification at p. 12, last paragraph), or with randomization and selection (specification at p. 21, lines 20-25). Thus, the claim specifies two design rules that can be used to select amino acids at one position in a zinc finger, to bind to one nucleotide of a quadruplet; but is additionally open to several possibilities, disclosed by the specification, for design or selection of amino acids (at other positions in the zinc finger) to bind other nucleotides in the quadruplet of bases recited in the claim. Accordingly, applicants believe that claim 1 is definite, and that the rejection can be withdrawn.

Second, the office action states that claim 1 is indefinite in that it does not produce the intended effect of preparing a nucleic acid binding protein. Although applicants believe that one of skill in the art, reading claim 1, would realize that the purpose of the design rules set out in claim 1 is for synthesis of a binding protein, claims 1 and 3 have been amended to recite specific method steps, solely to advance prosecution. Accordingly, this rejection can be withdrawn.

The office action also states that claims are indefinite for reciting "capable of binding." This phrase has been changed as requested by the Examiner. Of course, a zinc finger protein can only bind to its target when so contacted with the target, and it is not applicants' intent to limit the claims to situations in which zinc finger proteins are in contact and bound with their targets. However, it is believed that this intent is still clear from the amended claims. In light of the amendments, the rejection can be withdrawn.

Claim 4 was rejected for reciting "the or each" zinc finger. "Or each" has been deleted mooting the rejection.

Claims 4-12, 14 and 18 were rejected for the use of a one-letter amino acid code. These claims have been amended to recite amino acids in a three-letter code, as requested. Accordingly, this rejection can be withdrawn.

Claims 15 and 16 have been rejected as allegedly indefinite for the use of literature references rather than actual sequences. In response, applicants have deleted the references from claim 15. It is noted that the same references are provided in the specification at paragraph bridging pp 11-12. The zinc finger proteins referred to in claims 15 and 16 are well known naturally-occurring zinc finger proteins, and are referred to by names such as Zif268, GLI and so forth. These names are so frequently used in the scientific literature as to have become a convention in the field. For example, a quick search of the MedLine database indicates over 1100 scientific articles referring to Zif268. Accordingly, it is submitted that reference to these proteins by their conventional names in the claims is sufficient to identify them to those of skill in the art.

Further, applicants note that claims 19-21 are in improper multiple dependent form. In response, claim 19 has been amended to remove improper multiple dependency. Accordingly, this rejection of claim 19, and its dependent claims 20 and 21, can be withdrawn.

Claims 19-22 stand rejected as indefinite for depending from rejected base claims. The base claims have been amended as described above. Thus, the rejection can be withdrawn from claims 19-22 for the same reasons as for the base claims.

12. The office action states that, although Choo *et al.*, WO 96/06166 would anticipate claims 1-3 and 27 (sic, 1-24), a formal art rejection has not been applied due to the alleged indefiniteness of the claims. In support, the office action states that, when read in a 3'-5' direction, base 4 of a quadruplet is the equivalent of the 5' base (*i.e*, base 1) of an adjacent quadruplet. The office action also states that Choo recites the same

rules as recited in the present claims. Applicants respectfully traverse insofar as the Examiner's comments might be applied to the amended claims.

The present claims specify selection of a quadruplet of bases for design of a zinc finger and provide rules for each of the four bases in such a quadruplet. By contrast, Choo specifies selection of a triplet of bases and provides rules for the three bases in such a triplet. Whether a quadruplet of bases or a triplet of bases is chosen as the target site can have significant consequences on the sequence of the zinc finger(s) that result from following the respective rules in the present claims and those in the cited Choo case. Fig. 1 (attached) provides a simple example to illustrate the different consequences depending on which strategy is chosen. The figure illustrates design of one finger within a three-finger zinc finger protein in which the finger being designed is the first or N-terminal finger of the protein. The upper part of the figure shows the finger being designed according to the triplet selection strategy of Choo. The lower part of the figure shows the finger being designed according to the quadruplet selection strategy of the present claims. In both cases, the finger is intended to bind the same region of a target sequence. In the upper part of the figure only bases 2, 3 and 4 are used in the design process. In the lower part of the figure each of bases 1, 2, 3 and 4 are used. In this situation, base 1 of a quadruplet is not the equivalent of base 1 of a triplet in Choo's rules because there is no triplet adjoining the 3' end of the quadruplet that is used in design of a zinc finger. In consequence, applying the triplet or quadruplet rules leads to different results. When applying the quadruplet rules, base 1 of the quadruplet leads to selection of a glu at position +2 of finger 1. When applying the triplet rules, no account is taken of the "G" constituting base 1 of the quadruplet and position +2 of the first finger is not specified by the rules.

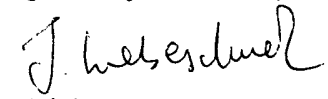
Fig. 2 (attached) illustrates that the quadruplet and triplet rules can also lead to different consequences in design of a zinc finger occupying the central position of a zinc finger. Again, the upper part of the figure shows design of a finger according to the triplet rules and the lower part of the figure according to the quadruplet rules. In the

lower part of the figure base "G" is shown twice because it occurs as the first base in one quadruplet and the fourth base in an overlapping quadruplet. Once again, the rules of the cited Choo reference and the presently-claimed rules can lead to different results. In the triplet scheme (*i.e*, the Choo rules), the 5' G shown under finger 1 specifies that position +6 of finger 1 is Arg, Ser or Thr, and if either of the latter then position +2 of the central finger is occupied by Asp. The 3' A of the triplet under finger 2 specifies that position –1 of the second finger is Gln and positions +2 of the central finger is Ala. These rules can be simultaneously satisfied only when position +6 of the first finger is Arg and position +2 of the middle finger is Ala. In the presently-claimed quadruplet scheme, position +2 of the central finger is occupied by Glu.

These examples illustrate that whether a quadruplet site or a triplet site is selected can make a significant difference to the composition of the zinc finger designed to bind to the site. Moreover, use of the quadruplet site is generally advantageous in that more bases are taken into account in the design. It is therefore submitted that the claimed step of selecting a quadruplet of bases renders the claims both novel and nonobvious.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claim 1 was amended as follows:

1. (Amended) [A method for preparing a nucleic acid binding protein of the Cys2-His2 zinc finger class capable of binding to a nucleic acid quadruplet in a target nucleic acid sequence, wherein binding to base 4 of the quadruplet by an α-helical zinc finger nucleic acid binding motif in the protein is determined as follows:]

A method for preparing a nucleic acid binding protein that binds to a target nucleotide sequence, wherein the binding protein comprises a plurality of zinc fingers of the Cys2-His2 class, wherein the method comprises:

i) selecting a quadruplet within the target nucleotide sequence;

ii) designing the binding protein such that binding of a zinc finger to the quadruplet is obtained by choosing the sequence of particular residues of the zinc finger depending on the nucleotide sequence of the quadruplet, as follows:

a) if base 4 in the quadruplet is A, then position +6 in the α-helix is Glu, Asn or Val;

b) if base 4 in the quadruplet is C, then position +6 in the α-helix is Ser, Thr, Val, Ala, Glu or Asn

iii) synthesizing a polynucleotide encoding the binding protein of (ii);

iv) introducing the polynucleotide of (iii) into a cell; and

v) incubating the cell under conditions in which the encoded nucleic acid binding protein is expressed.

Claim 2 was amended as follows:

2. (Amended) A method according to claim 1, wherein binding to base 4 of the quadruplet by [an α-helical] a zinc finger [nucleic acid binding motif in the protein] is additionally determined as follows:

c) if base 4 in the quadruplet is G, then position +6 in the α-helix is Arg or Lys;

d) if base 4 in the quadruplet is T, then position +6 in the α-helix is Ser, Thr, Val or Lys.

Claim 3 was amended as follows:

3. (Amended) [A method for preparing a nucleic acid binding protein of the Cys2-His2 zinc finger class capable of binding to a nucleic acid quadruplet in a target nucleic acid sequence, wherein binding to each base of the quadruplet by an α-helical zinc finger nucleic acid binding motif in the protein is determined as follows:]

A method for preparing a nucleic acid binding protein that binds to a target nucleotide sequence, wherein the binding protein comprises a plurality of zinc fingers of the Cys2-His2 class, wherein the method comprises:

i) selecting a quadruplet within the target nucleotide sequence;

ii) designing the binding protein such that binding of a zinc finger to the quadruplet is obtained by choosing the sequence of particular residues of the zinc finger depending on the nucleotide sequence of the quadruplet, as follows:

a) if base 4 in the quadruplet is G, then position +6 in the α-helix is Arg or Lys;

b) if base 4 in the quadruplet is A, then position +6 in the α-helix is Glu, Asn or Val;

c) if base 4 in the quadruplet is T, then position +6 in the α-helix is Ser, Thr, Val or Lys;

d) if base 4 in the quadruplet is C, then position +6 in the α-helix is Ser, Thr, Val, Ala, Glu or Asn;

e) if base 3 in the quadruplet is G, then position +3 in the α-helix is His;

f) if base 3 in the quadruplet is A, then position +3 in the α-helix is Asn;

g) if base 3 in the quadruplet is T, then position +3 in the α-helix is Ala, Ser or Val; provided that if it is Ala, then one of the residues at -1 or +6 is a small residue;

h) if base 3 in the quadruplet is C, then position +3 in the α-helix is Ser, Asp, Glu, Leu, Thr or Val;

i) if base 2 in the quadruplet is G, then position -1 in the α-helix is Arg;

j) if base 2 in the quadruplet is A, then position -1 in the α-helix is Gln;

k) if base 2 in the quadruplet is T, then position -1 in the α-helix is His or Thr;

l) if base 2 in the quadruplet is C, then position -1 in the α-helix is Asp or His;

m) if base 1 in the quadruplet is G, then position +2 is Glu;

n) if base 1 in the quadruplet is A, then position +2 Arg or Gln;

o) if base 1 in the quadruplet is C, then position +2 is Asn, Gin, Ara, His or Lys;

p) if base 1 in the quadruplet is T, then position +2 is Ser or Thr

iii) synthesizing a polynucleotide encoding the binding protein of (ii);

iv) introducing the polynucleotide of (iii) into a cell; and

v) incubating the cell under conditions in which the encoded nucleic acid binding protein is expressed.

Claim 4 was amended as follows:

4. (Amended) A method according to any preceding claim, wherein the [or each] each zinc finger has the general primary structure

[(A) X^a C X_{2-4} C X_{2-3} F X^c X X X X L X X **H** X X X^b **H** - linker (SEQ ID NO: 3)

-1 1 2 3 4 5 6 7 8 9]

X^a Cys X_{2-4} Cys-X_{2-3}-Phe-X^c-X-X-X-X-Leu-X-X-His-X-X-X^b His-linker (SEQ ID NO: 3)

-1 1 2 3 4 5 6 7 8 9

wherein X (including X^a, X^b and X^c) is any amino acid.

Claim 5 was amended as follows:

5. (Twice Amended) A method according to claim 4 wherein [X^a is F/Y-X or P-F/Y-X] Xa is Phe/Tyr-X or Pro-Phe/Tyr-X.

Claim 6 was amended as follows:

6. (Twice Amended) A method according to claim 5 wherein X_{2-4} is selected from any one of:

[S] Ser-X, [E] Glu-X, [K] Lys-X, [T] Thr-X, [P] Pro-X and [R] Arg-X.

Claim 7 was amended as follows:

7. (Twice Amended) A method according to claim 4 wherein X^b is [T] Thr or [I] Ile.

Claim 8 was amended as follows:

8. (Twice Amended) A method according to claim 4 wherein X^{2-4} is [G-K-A, G-K-C, G-K-S, G-K-G, M-R-N or M-R] Gly-Lys-Ala, Gly-Lys-Cys, Gly-Lys-Ser, Gly-Lys-Gly, Met-Arg-Asn or Met-Arg.

Claim 9 was amended as follows:

9. (Twice Amended) A method according to claim 4 wherein the linker is [T-G-E-K] Thr-Gly-Glu-Lys (SEQ ID NO: 4)or [T-G-E-K-P] Thr-Gly-Glu-Lys-Pro (SEQ ID NO: 5).

Claim 10 was amended as follows:

10. (Twice Amended) A method according to claim 4 wherein position +9 is [R or K] Arg or Lys.

Claim 11 was amended as follows:

11. (Twice Amended) A method according to claim 4 wherein positions +1. +5 and +8 are not occupied by any one of the hydrophobic amino acids, [F, W or Y] Phe, Trp or Tyr.

Claim 12 was amended as follows:

12. (Amended) A method according to claim 11 wherein positions +1, +5 and +8 are occupied by the residues [K, T and Q] Lys, Thr and Gln respectively.

Claim 13 was amended as follows:

13. (Amended) A method for preparing a nucleic acid binding protein of the Cys2-His2 zinc finger class [capable of binding to] which binds a target nucleic acid sequence, comprising the steps of:

a) selecting a model zinc finger domain from the group consisting of naturally occurring zinc fingers and consensus zinc fingers; and

b) mutating the finger according to the rules set in any one of claims 1 to 3.

Claim 14 was amended as follows:

14. (Twice Amended) A method according to claim 13, wherein the model zinc finger is a consensus zinc finger whose structure is selected from the group consisting of the consensus structure [P Y K C P E C G K S F S Q K S D L V K H Q R T H T G] Pro-Tyr-Lys-Cys-Pro-Glu-Cys-Gly-Lys-Ser-Phe-Ser-Gln-Lys-Ser-Asp-Leu-Val-Lys-His-Gln-Arg-Thr-His-Thr-Gly (SEQ ID NO: 6), and the consensus structure [P Y K C S E C G K A F S Q K S N L T R H Q R I H T G E K P] Pro-Tyr-Lys-Cys-Ser-Glu-Cys-Gly-Lys-Ala-Phe-Ser-Gln-Lys-Ser-Asn; Leu-Thr-Arg-His-Gln-Arg-Ile-His-Thr-Gly-Glu-Lys-Pro (SEQ ID NO: 7).

Claim 15 was amended as follows:

15. (Amended) A method according to claim 13 wherein the model zinc finger is a naturally-occurring zinc finger whose structure is selected from one finger of a protein selected from the group consisting of Zif 268 [(Elrod-Erickson *et al.*, (1996) Structure 4:1171-1180)], GLI [(Pavletich and Pabo, (1993) Science 261:1701-1707)], Tramtrack [(Fairall *et al.*, (1993)Nature 366:483-487)] and YY1 [(Houbaviy *et al.*, (1996) Proc. Natl. Acad. Sci. USA (USA) 93:13577-13582)].

Claim 18 was amended as follows:

18. (Twice Amended) A method according to claim 14, wherein the N-terminal zinc finger is preceded by a leader peptide having the sequence [MAEEKP] Met-Ala-Glu-Glu-Lys-Pro (SEQ ID NO: 8).

Claim 19 was amended as follows:

19. (Twice Amended) A method according to [claim 14] claim 13 wherein the nucleic acid binding protein is [constructed] obtained by recombinant nucleic acid technology, the method comprising the steps of:

a) preparing a nucleic acid coding sequence encoding two or more [zinc finger binding motifs as defined in claim 13] model zinc finger domains, placed N-terminus to C-terminus;

b) inserting the nucleic acid sequence into a suitable expression vector; and

c) expressing the nucleic acid sequence in a host organism in order to obtain the nucleic acid binding protein.

Claim 22 was amended as follows:

22. (Amended) A method according to claim 21, comprising the steps of:

a) preparing a nucleic acid construct [capable of expressing] which express a fusion protein comprising the nucleic acid binding protein and a minor coat protein of a filamentous bacteriophage;

b) preparing further nucleic acid constructs [capable of expressing] which express a fusion protein comprising a selectively mutated nucleic acid binding protein and a minor coat protein of a filamentous bacteriophage;

c) causing the fusion proteins defined in steps (a) and (b) to be expressed on the surface of bacteriophage transformed with the nucleic acid constructs;

d) assaying the ability of the bacteriophage to bind the target nucleic acid sequence and selecting the bacteriophage demonstrating superior binding characteristics.